ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN VITRO ANTIOXIDANT ACTIVITY OF TRIGONELLA FOENUM-GRACEUM LINN.

Botany

Kumari Ramona Research scholar, Department of Botany, Magadh University, Bodh Gaya-824234.

Baidyanath Kumar* Academic Director, Life Science Research Centre, Patliputra, Patna-800001.
*Corresponding Author

ABSTRACT

Background: Trigonella founum-graceum is a medicinally important plant used in the treatment of loss of appetite, flatulence, dyspepsia, colic, diarrhoea, dysentery; enlargement of liver and spleen, oxidative stress and diabetes. Seeds contain alkaloids, saponins, flavonoids and volatile oil as phytochemicals. **Aim:** To study the antioxidant activities of seeds. **Objectives:** To study the reducing power and DPPH scavenging activities of methanol extracts of seeds. **Methods:** The antioxidant activities of seeds were assayed quantitatively in vitro using reducing power and scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical methods. **Results:** The methanol extract (0.5-1.5 mg/ml) and the standard antioxidant n-propyl gallate (0.003-0.03 mg/ml) caused a concentration – dependent reduction of Fe3+ to Fe2+. Similarly, the DPPH scavenging activity of the methanol extracts also exhibited concentration-dependent free radical scavenging activity. **Conclusions:** On the basis of the present results it can be concluded that the methanolic extracts seeds of Trigonella foenum-graceum possess significant antioxidant activity.

KEYWORDS

Trigonella Foenum-graceum, Methanol Extract, Seeds, Antioxidant

INTRODUCTION

Trigonella foenum-graceum Linn. (Fenugreek or Methi) of family Fabaceae is an erect, annual, aromatic herb, 30-45 cm high; Leaves are pinnately 3-foliolate; leaflets 2-2.5 cm long, oblanceolate-oblong toothed; Flowers are 1-2, axillary, sessile, corolla much exerted. Pod 5-7.5 cm long with a long persistent beak, often falcate, 10-20 seeded.

The seeds of this plant are used as traditional medicine in loss of appetite, flatulence, dyspepsia, colic, diarrhoea, dysentery; enlargement of liver and spleen, and as a lactagogue and puerperal tonic. The seeds are known to possess secretolytic, hyperemic and mild antiseptic, demulcent and hypoglycaemic activities.

The important phytochemicals of seeds include alkaloids (trigonelline, gentianine and carpaine), saponins (sapogenins, diosgenin, yamogenin, gitogenin and tigogenin), flavonoids (vitexin and and luteolin) and a volatile oil in small quantities. The mucilage is mostly a galactomannan. The peptide ester of steroidal sapogenin viz. fenugreekine exhibits hypoglycaemic activity.

Oxidation process results in the production of free radicals which abruptly interfere with the normal metabolic activities of the body causing immense damage to the normal tissues[1]. There is a close relationship between diabetes and oxidative stress and it has been reported that the free radicals are produced in the form of ROS (reactive oxygen species) which cause mutation in mitochondrial DNA thus resulting in hypoglycemic memory[2]. Free radicals interfere with vital organ and may lead to cardiovascular complications, diabetic nephropathy, diabetic retinopathy, erectile dysfunction and diabetic neuropathy[3]. Several plants are known for their efficacy to overcome these complications by enhancing the *in vivo* anti oxidant defense and provide protection against oxidative tissue damage[4]. The SOD (Superoxide dismutase), CAT (Catalase), Vitamin E and C are some of the antioxidants which provide protection to the tissues[5]and their level of defense can be assessed by measuring the MDA concentration which is the end product of lipid peroxidation[6].

All cells in eukaryotic organisms contain powerful antioxidant enzymes. Endogenous antioxidants made in the body are believed to be more potent in preventing free radical damage than exogenous antioxidants. The major classes of endogenous antioxidant enzymes are the superoxide dismutases, catalases and glutathione peroxidases[1], α-lipoic acid and coenzyme Q10. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds.

In the present investigation antioxidant activity of methanol extract of seeds of *Trigonella foenum-graceum* was assayed *in vitro.*

Materials and Methods

The fresh seeds of *Trigonella foenum-graceum* were washed under running tap water, blotted with filter paper and dried in shade at room temperature for 7 days. The dried seeds were powdered using a mixture grinder and stored in air-tight container for future use. Methanol was used for preparation of solvent extracts. The dried sample was soaked separately with methanol under reflux condition for the solvent extract preparation. About 1 gm of the dried sample was added into the test tubes containing 5 ml of solvent and was extracted at room temperature. The sample was homogenized with extraction buffer. The supernatant was collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40^0C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The methanol extracts thus obtained were then stored separately in deep freezer at -20^0C until further test. The antioxidant activity of methanol extract was assayed quantitatively by following two methods:

1. Reducing power assay: Reducing activity of methanol extract was assayed as follows. Different concentrations (0.25 – 2.0 mg/ml) of the extracts as well as the standard drug *n*-propyl gallate (3.75 – 30 µg/ml) were prepared in aqueous methanol (50% v/v) and 1 ml each taken into test tubes in triplicates. To the test tubes, 2.5 ml of sodium phosphate buffer (pH=7) and 2.5 ml of 1% potassium ferric cyanide solution was added. The contents were mixed well and incubated at 50 °C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 3000 revolutions per minute for 10 minutes. After centrifugation 2.5 ml of supernatant was added to 2.5 ml of distilled water. To this about 1 ml of 0.1% ferric chloride was added. The absorbance was then taken at 700 nm. A graph of absorbance was then plotted against the concentration of the extracts. Increase in absorbance indicates higher reducing power of the extract.

2. Scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay: The free radical scavenging activity was determined as follows. 1 ml each of the extracts (0.25, 0.5, 1.0 and 2 mg/ml in methanol) was added to 3 ml methanolic solution of DPPH solution (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for 30 mins. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). One milliliter (1 ml) methanol (50%) was added to 3 ml DPPH solution, incubated at 25 °C for 30 minutes and used as control. *n*-propyll gallate (3.75-30 µg/l) was used as a standard free radical scavenger. The absorbance decreases with increasing free radical scavenging ability. Results were expressed as percentages of blank (100%). The concentration required to cause a 50% decrease in the absorbance was calculated (EC50). Each test was carried out using three replicates. The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows:

$$\%\,\text{DPPH scavenging effects} = (Ac - At)/Ac \times 100$$

Where

Ac = Absorbance of the control
At = Absorbance of the test drug/ extracts

RESULTS

1. Reducing power

The methanol extract (0.5-1.5 mg/ml) and the standard antioxidant *n*-propyl gallate (0.003-0.03 mg/ml) caused a concentration – dependent reduction of Fe3+ to Fe2+. From the IC50 values (Table-1), the methanol extract of seeds showed the highest reducing power activity. At 1.5 mg/ml of the methanol extract the IC50 value were found to be maximum i.e. 575.35 µg/ml

Table-1: Reducing power of methanol extracts seeds of *Trigonella foenum-graceum*

Dose of methanol extract in mg/ml	T. foenum-graceum IC50 (µg/ml) ±SEM
0.5	190 ±0.025
1.0	235.15 ±0.016
1.5	575.35±0.12
n-PG	70.25±0.003

2. Free Radical Scavenging Activity

The results of the free radical scavenging potential of methanol extracts of seeds of *Trigonella foenum-graceum* using DPPH free radical scavenging method have been presented in Table-2. The DPPH scavenging activity of the methanol extracts (0.5 -1.5 mg/ml) also exhibited concentration-dependent free radical scavenging activity. The methanol extract of seeds of *Trogonella foenum-graceum* exhibited highest free radical scavenging activity (IC50 value 580.7µg/ml) at concentration of 1.5 mg/ml.

Table-2: DPPH scavenging activity of methanol extracts of *seeds of Trigonella foenum-graceum*

Dose of extract in mg/ml	F. microcarpa IC50 (µg/ml) ±SEM
0.5	85.5 ±0.025
1.0	250.5 ±0.095
1.5	580.7 ±0.087

DISCUSSION:

In the present investigation, antioxidant activity of the methanol extracts of seeds of *Trigonella foenum-graceum* were assayed by reducing power and DPPH scavenging activities. In both these assays, the antioxidant activity increased with increasing concentration of the methanol extract.(Table-1and Table-2). The total methanol extract showed higher reducing power activity at concentration of 1.5 mg/ml which is considerably higher than the standard antioxidant *n*-propyl gallate (IC50 = 70.25± 0.003 µg/ml).

Phytochemicals such as flavoniods, alkaloids, tannins and other aromatic compounds are secondary metabolites that are known to serve as defense mechanisms. The presence of pharmacologically active phytochemical in methanolic extracts of seeds of *Trigonella foenum- graecum* has been reported by several workers[7,8,9] that possess antioxidant, antimicrobial and hypoglycemic activities. More or less similar antioxidant activities have also been reported by Estevinho *et al*[10],Vijay Kumar and Bhat[11], Kaviarasan *et al*[12], Losso *et al*[13], Adedapo *et al*[14] and Emtiazy *et al*[15] for seeds of fenugreek.

CONCLUSIONS:

Plants are sources of new natural products used in pharmaceutical, cosmetic and food production. An *in vitro* antioxidant assay provides scientific evidence to prove the traditional claims to the *Trogonella foenum-graceum as* medicinal values. On the basis of the present results it can be concluded that the methanolic extracts seeds of this plant possess significant antioxidant activity. The present investigation suggests that seeds of fenugreek are a potential source of natural antioxidant. The active principles responsible for antioxidant activity and their mechanism of action *in vivo* as well as *in vitro* require further investigation at scientific level.

ACKNOWLEDGEMENT

Authors are thankful to Dr. D. K. Yadav, Retd. Professor and head, Department of Botany, Magadh University, Bodh Gaya for providing necessary suggestion and support.

REFERENCES

[1]. Sies, H. (1997): Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, 82: 291-295.
[2]. Davy G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, *et al*., (1999): *In vivo* formation of 8-iso-prostaglandin F2a and platelet activation in diabetes mellitus, effect of improved metabolic control and Vitamin E supplementation. Circulation **99**, 224-229.
[3]. Ann MS, David S, (2000): A radical approach to the pathogenesis of diabetic complications. *TiPS* 21, 367-369.
[4]. Roja R, Shekoufeh N, Bagher L,Mohammad A.(2005): A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine & Pharmacotherapy* 59, 365–373.
[5]. Durdi Q, Masomeh H, Hamideh D, Timur R.(2005): Malondialdehyde and carbonyl contents in the erythrocytes of streptozotocin-induced diabetic rats. *Int. J. Diabetes & Metabolism* 13, 96-98.
[6]. Hostetter TH, Troy JL, Brenner BM.(1981): Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int*. **19**, 410–415.
[7]. Mandegary A, Pournamdari M, Sharififar F, Pournourmohammadi S, Fardiar R and Shooli S. (2012): Alkaloid and flavonoids rich fraction of Fenugreek seeds (*Trigonella fenum-graecum* L.) with antini ciceptive and anti-inflamatory effects. *Food and chemical toxicology*, 50(7): 2503-2507.
[8]. Tsao R, Deng Z. (2004): Separation procedures for naturally occurring antioxidant phytochemicals. J. Chromatogr. B Analyt. Technol. Biomed. *Life Sci*., 812: 85-99.
[9]. Bukhari SB, Bhanger MI, Memon S. (2008): Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum). Pak. J. Anal. Environ. Chem.,* 9: 78-83.
[10]. Estevinho L, Pereira AP, Moreiral G, Pereira E. (2008): Antioxidant and antimicrobial effects of phenolic compounds extracts of northeast Portugal honey. *Food Chem Toxicol.* 46:3774–9.
[11]. Vijayakumar MV, Bhat MK. (2008):Hypoglycemic effect of a novel dialyzed fenugreek seeds extract is sustainable and is medicated, in part, by the activation of hepatic enzymes. *Phytother Res*. 22(4):500–50.
[12]. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha VC, Priyadarsin IK. (2007): In vitro studies on antiradical and antioxidant activities of fenugreek seeds. *Food chem.* 103:31–7.
[13]. Losso JN, Holiday DL, Finley JW, *et al*., (2009): Fenugreek bread: a treatment for diabetes mellitus. *J Med Food*. 12(5):1046–9.
[14]. Adedapo AA, Ofuegbe OS, Soetan OK. (2014): Pharmacologic and medicinal properties of fenugreek. *Am J Soc Sci Issues Hum. (*Special issue) 13–20.
[15]. Emtiazy, M., Oveidzadeh, L., Habibi, M. *et al.,* (2018): Investigating the effectiveness of the *Trigonella foenum-graecum* L. (fenugreek) seeds in mild asthma: a randomized controlled trial. *Allergy Asthma Clin Immunol* 14, 19, https://doi.org/10.1186/s13223-018-0238-9